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Strain Difference in Free p-Cresol Excretion in Urine of Rats Exposed to Toluene at Sub-narcotic Concentrations

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Since the observation on strain difference in toluene metabolism in rats (Inoue et al. 1984), evidence has been accumulating in this laboratory to suggest that possibly there is an ethnic-related difference in organic solvent metabolism in humans. A significant difference in o-cresol/hippuric acid ratio after exposure to toluene was first observed among the four strains of rats (Inoue et al. 1984), and its presence was further confirmed also in workers of various ethnic backgrounds (Inoue et al. 1986, and 1988a and b). Namely, under comparable intensities of exposure to toluene, the urinary hippuric acid levels were higher among Japanese workers than Chinese workers, and the o-cresol/hippuric acid ratio also varied among Japanese, Chinese and Turkish workers (Inoue et al. 1986). Similarly, the exposure - excretion relationship was different among Japanese, Chinese and Korean toluene-exposed workers (Inoue et al. 1988a), and between Japanese and Chinese workers exposed to trichloro-ethylene (Inoue et al. 1988b).

Analyses were made for additional evidence on strain difference in animals to consolidate the observation in workers, and it was found that free p-cresol excretion varied in rats of various strains exposed to toluene. The finding is to be presented in this report. The observation on total p-cresol has been previously reported (Inoue et al. 1984) and will be discussed also in this report as necessary.

MATERIALS AND METHODS.

Female rats of Donryu, Fischer, Sprague-Dawley and Wistar strains (6-7 week-old with mean body weights of

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183, 136, 184 and 143 g, respectively) were used. The animals, 4-8 per group and housed individually in each metabolic cage, were exposed to toluene at 0 (sham-exposed), 5, 45, 500, 2500 and 3500 ppm for 8 hours in a dynamic flow-type exposure system (Koizumi and Ikeda 1981; Kumai et al. 1984). Urine samples of individual rats were collected separately from feces for 24 hours from the initiation of the exposure, during which period more than 95% of the toluene metabolites were excreted into urine (Ikeda and Ohtsuji 1971). The urine samples which had been analyzed for hippuric acid and o-cresol (Inoue et al. 1984) were employed. p-Cresol in urine was determined with (i.e., heated for 60 min at 100°C in the presence of 0.5N HCl) and without (i.e., solvent extraction immediately after the addition of HCl at a final concentration of 0.25 N) acid hydrolysis followed by the gas-chromatography as described previously (Inoue et al. 1984) with 3,5-xylenol as an internal standard. The amount was expressed in terms of μg free (i.e., unconjugated) p-cresol/kg body weight excreted in the 24 hour period. The amount determined without the hydrolysis was taken as free (unconjugated) p-cresol, and that after hydrolysis as total (i.e., the sum of free and conjugated) p-cresol.

For statistical evaluation, the p-cresol concentrations were considered to distribute log-normally, and the analysis of variance (ANOVA) was employed for the detection of significant difference in results, unless otherwise specified.

RESULTS AND DISCUSSION.

Of the results of p-cresol determinations in the urine of the four strains of rats, free p-cresol levels are depicted in Figure 1 in terms of geometric means and geometric standard deviations. The toluene levels in air and the free p-cresol concentrations in urine are taken on the ordinate and the abscissa, respectively, both after logarithmic conversion.

Although the variation in free p-cresol excretion was wide among the rats of the same strain exposed to toluene at the same concentration (as shown by long arrows for the geometric standard deviations in Figure 1), it was also evident from Figure 1 that the four strains tested may be classified into two groups depending on the free p-cresol levels at the toluene concentrations of 0 to 500 ppm. For example, there was no significant difference ($P>0.10$ by t-test) in free p-cresol levels between Sprague-Dawley and Wistar strains, nor between Donryu and Fischer strains at the toluene levels of 45 and 500 ppm. When Sprague-Dawley

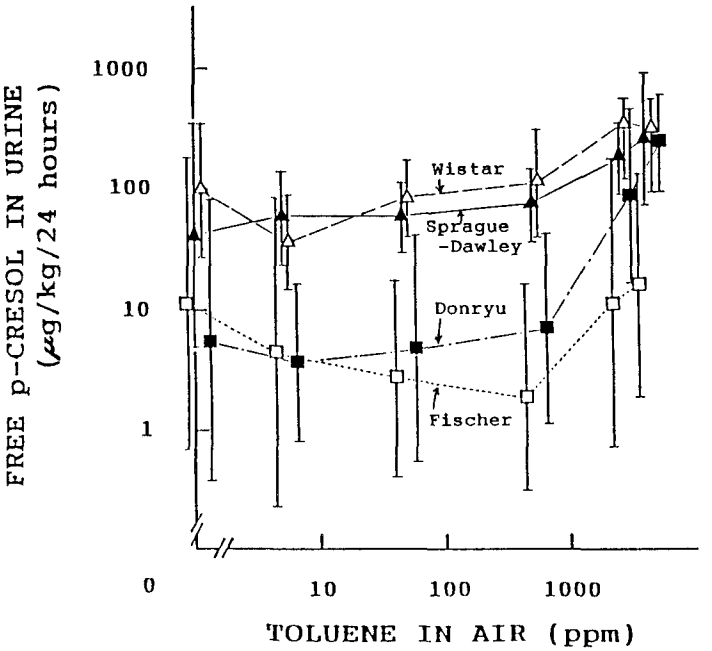

Figure 1. Urinary excretion of free p-cresol after exposure to toluene at various concentrations. Four strains (Sprague-Dawley, solid triangles with solid lines; Wistar, open triangles with broken lines; Donryu, solid squares with dotted broken lines; Fischer with open squares with dotted lines) of rats, 4 to 8 animals per strain and per concentration, were exposed for 8 hours to toluene at 0, 5, 45, 500, 2500 and 3500 ppm. Urine samples were collected from individual rats for 24 hours from the initiation of the exposure. Geometric means (symbols) and geometric standard deviations (arrows) of free p-cresol are depicted against toluene concentration on the double logarithmic scales.

rats were combined with Wistar rats, and Donryu rats with Fischer rats, there was a significant ($P<0.05$) difference between the two combinations at any of the two toluene exposure levels. Thus, the former two would form one group with high p-cresol excretion, and the latter two would form the other with low p-cresol excretion. At the higher toluene concentrations of 2500 and 3500 ppm, however, the increase in the free p-cresol level was remarkable only in Donryu rats so that the level in Donryu rats reached the levels of Sprague-Dawley and Wistar rats, while the level in Fischer rats remained essentially unchanged. The ANOVA with an assumption of log-normal distribution disclosed that there was no effect of toluene exposure on free p-

cresol excretion in Fischer strain ($P<0.05$), while the effect was significant ($P<0.01$) in Donryu strain in addition to Sprague-Dawley and Wistar strains. When the distribution was considered to be normal, the effect was significant ($P<0.01$) only in Donryu rats but not ($P>0.05$) in any of other three strains.

It has been shown in vivo that toluene given to mammals will be primarily oxidized at the side chain and then conjugated with glycine to be excreted into urine as hippuric acid (El Masry et al. 1956), while it will also be oxidized at the aromatic ring to a lesser extent to be cresols which will mostly undergo sulfate or glucuronide conjugation before urinary excretion (Bakke and Scheline 1970; Woiwode et al. 1979; Woiwode and Drysch 1981). When 100 mg/kg of toluene was given by mouth to rats, 0.4 to 1.0 % and 0.04 to 0.11 % of the dose were excreted in urine as p- and o-cresol, respectively (Bakke and Scheline 1970). p-Cresol was most abundant in urine of men experimentally exposed to toluene, followed by o-cresol and then m-cresol among the three isomers of cresols (Woiwode et al. 1979; Woiwode and Drysch 1981). Thus, p-cresol is apparently a minor yet second leading metabolite of toluene both in man and in rats.

A significant inter-strain difference ($P<0.01$) was observed in the preceding study (Inoue et al. 1984) among the four strains of rats in the ratio of o-cresol over hippuric acid in urine when the rats were exposed to toluene at high concentrations of 2500 or 3500 ppm, while the amount of hippuric acid per body weight was essentially the same among the four strains. In addition to this finding on inter-strain difference in toluene metabolism, the present study made it clear that the inter-strain difference also exists in the exposure - excretion relationship in the amount of urinary free p-cresol.

As for the levels of total p-cresol in the urine of the four strains of rats (the values cited from a previous publication; Inoue et al. 1984), an exposure-dependent increase was significant in any of the four strains ($P<0.01$ for each by ANOVA). It was observed in the present study that free p-cresol levels clearly increased in Donryu rats, not very much in Sprague-Dawley and Wistar rats, and stayed unchanged in Fischer rats, when the animals were exposed to toluene up to 3500 ppm (Figure 1). The observation then implies that the capacity to conjugate p-cresol is sufficient in the three strains of Sprague-Dawley, Wistar and Fischer to cope with toluene exposure-induced increase in p-cresol production, but not enough in Donryu strain so that free p-cresol will be excreted after intensive exposure

Table 1. Free p-cresol/total p-cresol ratio (%) in four strains of rats exposed to toluene at various levels

Strain	Toluene in ppm (No. of rats)			
	5 (4)	45 (8)	2500 (6)	3500 (6)
Sprague-Dawley	25.9 (19.7)	20.2 (14.5)	6.8 (5.9)	7.0 (3.9)
Wistar	4.2 (2.5)	7.2 (4.6)	5.1 (4.6)	4.1 (2.5)
Donryu	1.3 (0.8)	1.9 (0.6)	5.5 (2.6)	4.0 (2.6)
Fischer	10.5 (1.3)	2.1 (0.4)	2.4 (0.3)	0.5 (0.2)

The values in the table are arithmetic means (geometric means in parentheses) of free p-cresol/total p-cresol ratio in the urine collected as described under Figure 1. The animals were exposed for 8 hours.

(i.e., at 2500 to 3500 ppm for 8 hours) to toluene. In fact, the calculation for free p-cresol/total p-cresol ratio disclosed (Table 1) that, although the individual variation was wide, Donryu strain was the only strain in that the free p-cresol/total p-cresol ratio tended to increase in accordance with more intensive exposure to toluene, whereas the ratio either decreased (Sprague-Dawley and Fischer rats) or remained unchanged (Wistar rats) in other strains.

Inter-species differences have been reported both in sulfation and glucuronidation of phenol. Thus, pigs excrete mostly phenyl glucuronide and little phenyl sulfate when given phenol (Kao et al. 1979), in contrast to cats which have little capacity to excrete phenyl glucuronide after phenol administration (Capel et al. 1972) although both species of animals appear to have enough capacity to eliminate phenol as conjugated phenols. While no article has ever been published to report that the capacity of conjugation in the metabolism of industrial chemicals such as solvents may vary among different strains of a single animal species, the subject apparently deserves further attention in connection with possible ethnic difference in man as observed in the case of drug metabolism (e.g., Mahgoub et al. 1977; Eichelbaum et al. 1979).

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